In the Specification

Please replace the paragraph at page 3, lines 12 through 16 with the following paragraph:

B1 In another aspect of the present invention, a device that acts as a pre-column filter for reducing unwanted proteolysis on a chromatography column during purification of a target protein is provided. The pre-column filter is an affinity chromatography resin useful for removing proteases from crude protein extracts. In one embodiment, the pre-column filter comprises bovine lens alpha crystallin coupled to cyanogen bromide activated sepharose (CNBr-sepharose).

Please replace the paragraph at page 4, line 16 with the following paragraph:

B2 FIG. 3 is a digital image of a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE gel) showing purified BC-pepsinogen.

Please replace the paragraph at page 4, lines 18 through 23 with the following paragraph:

B3 FIG. 4 shows a digital image of a 12% SDS PAGE gel of p26 protein purified by nickel affinity chromatography resin. Because p26 is a multi-oligomer, it has a tendency to elute over several fractions, even when a sharp gradient is provided. Fractions identified using the SDS gel and containing p26 are dialyzed into Pipes magnesium buffer (20 mM piperazine-1,4-bis(2-ethanesulphonic acid (Pipes) pH 7.0, 1 mM $MgCl_2$). Following dialysis the target protein was stored at -20°C and used in less than 1 week for kinetic assays and chromatography experiments.

Please replace the paragraph at page 5 lines 13 through 26 with the following paragraph:

B4 Referring to FIG. 1, an expression vector consisting of a gene fusion between an unstable or insoluble protein could be stabilized or protected from proteolysis with the appropriate class of small molecular chaperone/alpha crystallin type proteins such as p26 from Artemia, SicA from Salmonella and alpha-A-crystallin protein from bovine lens. Unexpectedly it was shown that the protein p26 from *Artemia* has an active domain that can assist in the formation of soluble proteins based on its properties as an alpha-crystallin type protein. It was determined that full-length p26 protein is completely insoluble when express in *E. coli* at 37°C. It is expected that

proteins with a similar structural fold such as the SicA protein from *Salmonella typhimurium* could be substituted for p26. These results indicate that p26 and SicA are functionally very similar. It is also envisioned that the chaperone could be co-expressed on two different promoters either on the same plasmid or on different plasmids in the bacteria. The advantages of co-expression are that it would not require the removal of a fusion tag (such as thrombin) prior to purifying the protein of interest.

Please replace the paragraph at page 9, line 15 through page 10, line10 with the following paragraph:

The entire p26 protein was expressed by growing *E. coli* to an optical density of about 1.0 and then inducing 4 hours with 1 mM IPTG. P26 protein was purified with Ni^{2+}-NTA sepharose (Qiagen, cat #30410,) using the detergent DECAMEG (Calbiochem, cat# 373272, lot# b27260) to gently strip the protein contaminants that are nonspecifically bound to the p26 in the crude extract. At 37°C, all of the protein is in inclusion bodies, thus, an inclusion body prep was used to purify the protein. Briefly, following lysis by 3 x 15 sec bursts of sonication, the cell extract is centrifuged at 13 k revolutions per minute (RPM) for 15 minutes. The pellet was resuspended in low buffer (10 mM Tris pH 8.0, 500 mM NaCl) with 0.1% detergent (HECAMEG) with protease inhibitors (1.5 μg/ml leupeptin, 1.0 μM pepstatin, 0.2 μM phenylmethanesulfonyl fluoride (PMSF)). The insoluble protein was recentrifuged and the pellet was resuspended in low buffer with 1% detergent. After centrifuging the sample one time, the pellet was resuspended in 8M Urea. Low ionic strength buffer is used to bring the solution to 4 M urea, the sample was centrifuged again, and the supernatant was filtered through a 0.2 μm filter and loaded onto a nickel sepharose column. After loading the column, the p 26 was refolded with a linear reverse gradient of Urea (4-0 M Urea in low buffer). The protein bound to the column is washed with a 0.1% detergent to remove protein contaminants and the p26 is eluted with a linear gradient of high Imidazole buffer (10 mM Tris, 50 mM NaCl, 250 mM Imidazole). The protein fractions were run on a 12% SDS PAGE gel to determine which fractions were to be pooled. FIG. 4 shows a digital image of a 12% SDS PAGE gel of p26 protein purified by nickel affinity chromatography resin. Because p26 is a multi-oligomer, it has a tendency to elute overt several fractions, even when a sharp gradient is provided. Fractions identified to contain protein using

the SDS gel were dialyzed into Pipes-magnesium buffer (10 mM Pipes, PH 7.0, 1 mM $MgCl_2$, 50% glycerol). The high glycerol in the dialysis buffer concentrates the protein five times and the typical yield is about 5 mg/L. Following dialysis the target protein was stored at -20°C and used in less than 1 week for kinetic assays and chromatography experiments.

Please replace the Abstract with the following Abstract:

A method for expressing proteins as a fusion chimera with a domain of p26 or alpha crystallin type proteins to improve the protein stability and solubility when over expressed in bacteria such as *E. coli* is provided. Genes of interest are cloned into the multiple cloning site of the Vector System just downstream of the p26 or alpha crystallin type protein and a thrombin cleavage site. Protein expression is driven by a strong bacterial promoter (TAC). The expression is induced by the addition of 1 mM IPTG that overcomes the lac repression (lac I_q). The soluble recombinant protein is purified using a fusion tag.

Amendments to the specification and the Abstract are indicated in the attached "Marked Up Version of Amendments" (pages i - iii).

In the Claims

Please cancel Claims 1-5.

Please add new Claims 6-15.

6. (New) A method for producing soluble and active recombinant protein comprising:

a) expressing an insoluble protein as a fusion protein with an alpha-crystallin type protein or a fragment thereof comprising an active domain in bacteria;

b) purifying said fusion protein; and

c) removing said alpha-crystallin type protein or fragment thereof from said purified fusion protein,

thereby resulting in said soluble and active recombinant protein.